

pFUSE-CHlg-mA

Plasmid featuring the constant region of the mouse IgA heavy chain

Catalog code: pfuse-mcha

For research use only

Version 24J28-MM-v37

PRODUCT INFORMATION

Content:

- 20 µg of pFUSE-CHlg-mA plasmid provided as lyophilized DNA.
- 1 ml of Zeocin® (100 mg/ml)

Storage and Stability:

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20°C and is stable 3 months.
- Resuspended DNA should be stored at -20°C and is stable up to 1 year.
- Store Zeocin® at 4 °C or at -20 °C. The expiry date is specified on the product label.

Quality control:

- Plasmid construct has been confirmed by restriction analysis and sequencing.
- Plasmid DNA was purified by ion exchange chromatography.

Materials required for antibody generation & isotype switching

- pFUSE2-CLlg plasmid that features the constant region of the kappa or lambda light chains. pFUSE2-CLlg plasmids are selectable with blasticidin (sold separately, see RELATED PRODUCTS).
- pFUSE-CHlg plasmid for the constant region of the heavy chain, this plasmid is selectable with Zeocin®.

GENERAL PRODUCT USE

pFUSE-CLlg and pFUSE-CHlg plasmids are designed to change a monoclonal antibody from one isotype to another, therefore, enabling the generation of antibodies with the same antigen affinity but with different effector functions (increased or reduced ADCC and CDC). Furthermore, they can be used to produce entire antibodies from Fab or scFv fragments.

pFUSE-CHlg and pFUSE2-CLlg express the constant regions of the heavy (CH) and light (CL) chains, respectively. They contain a multiple cloning site (MCS) upstream of these constant regions to enable the cloning of the variable (VH and VL) regions of a given antibody. Transfection of mammalian cell lines with the recombinant pFUSE-CHlg and pFUSE2-CLlg pair allows to generate an antibody that can be purified from the supernatant using the appropriate affinity chromatography.

Features of pFUSE-CHlg and pFUSE2-CLlg plasmids

- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter¹ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat². The EF-1α promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1α core promoter to enhance stability of RNA.
- **MCS:** The multiple cloning site contains several restriction sites that are compatible with many other enzymes, thus facilitating cloning.
- **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA³.
- **ori:** a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.
- **CMV enh / hFerL prom:** This composite promoter combines the human cytomegalovirus immediate-early gene 1 enhancer and the core promoter of the human ferritin light chain gene. This ubiquitous promoter drives the expression of the Zeocin®-resistance gene in mammalian cells.
- **EM2KC** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*. EM2KC is located within an intron and is spliced out in mammalian cells.
- **βGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription⁴.

pFUSE-CHlg-mA specific features

- **Mouse IGHA (IgA heavy chain constant region):** When cloning your heavy chain variable region of choice in the MCS, care must be taken to insert the gene in-frame and to preserve the integrity of the heavy chain constant region.
- **Zeo:** Resistance to Zeocin® is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus*. The same resistance gene confers selection in both mammalian cells and *E. coli*.

References:

1. Kim DW. *et al.* 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. 91(2):217-23.
2. Takebe Y. *et al.* 1988. SR alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. Mol Cell Biol. 8(1):466-72.
3. Carswell S. & Alwine JC. 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol Cell Biol. 9(10):4248-58.
4. Yu J. & Russell JE. 2001. Structural and functional analysis of an mRNP complex that mediates the high stability of human beta-globin mRNA. Mol Cell Biol. 21(17):5879-88.

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com



PROTOCOL

Obtaining VH and VL sequences

The antibody sequence can be obtained by phage display or from an antibody producing hybridoma. To obtain the cDNA sequence of the VH and VL regions from an antibody producing hybridoma, total RNA or mRNA is extracted and reverse transcribed to cDNA. PCR is performed with 5' degenerate primers to anneal to the unknown VH and VL regions and the 3' primers designed to anneal to the "known" CH and CL regions. Alternatively 5' RACE can be used. The resulting amplicons must be sequenced.

Plasmid resuspension

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1 $\mu\text{g}/\mu\text{l}$, resuspend the DNA in 20 μl of sterile H₂O. Store resuspended plasmid at -20°C.

Cloning into pFUSE-CHIg and pFUSE2-CLIg

Once the VH and VL sequence are known, inserts for cloning into the plasmids can be generated. In pFUSE-CHIg-mA, the constant region of the mouse IgA heavy chain is preceded by a multiple cloning site containing four unique restriction sites: AgeI, EcoRV, XhoI, and NheI. The first three can be used for insertion of the 5' end of the variable region including the native signal sequence. If the immunoglobulin signal sequence is unknown, pFUSEss plasmids containing a signal sequence should be used. In pFUSE-CHIg-mA, NheI must be used for insertion of the 3' end of the variable region. NheI must be reconstituted to maintain the integrity of the constant region. Therefore we recommend to introduce by PCR an NheI site at the 3' end of the variable region in frame with the constant region.

When generating the insert for VL, a BstAPI (pFUSE2-CLIg-mk; mouse kappa), or AvrII (pFUSE2-CLIg-ml1 / pFUSE2-CLIg-ml2; mouse lambda) site must be introduced at the 3' end. There is a choice of restriction sites at the 5' end.

Note: The 5' end of the variable region should encompass the native ATG initiation codon and the region immediately after which corresponds to the signal sequence. For proper initiation of translation, make sure that your insert contains a Kozak translation initiation sequence upstream of the ATG initiation codon such as (G/A)NNATG.

Choice of strategies for the transfection

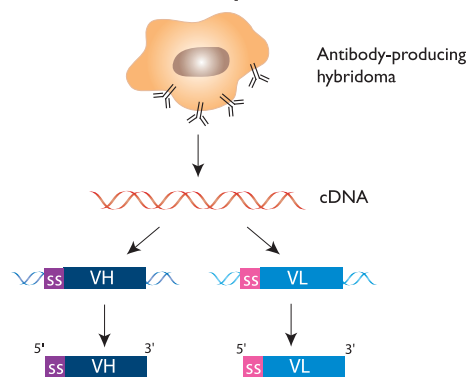
Transfect cells using a transfection agent, such as LyoVec™, with the plasmid coding for light chain and select the best clone. Following selection of the best clone, the plasmid coding for the heavy chain clone can be transfected into this clone.

OR

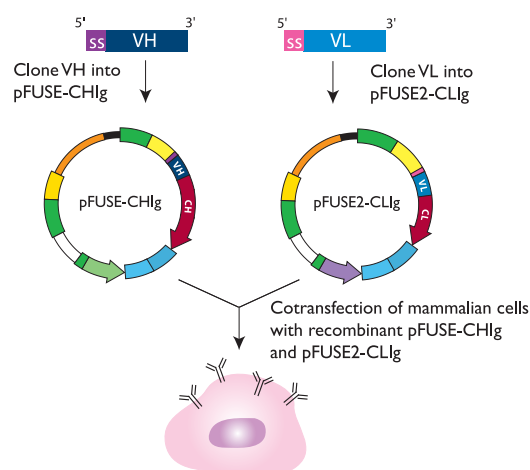
A cotransfection can be performed with the plasmid coding for the light chain and the plasmid coding for the heavy chain. Since the pFUSE2-CLIg and pFUSE-CHIg plasmids share the same plasmid backbone, the appropriate heavy chain to light chain ratio can be easily determined by varying the quantities of pFUSE2-CLIg and pFUSE-CHIg plasmids. We recommend using a ratio of 3:2 of pFUSE2-CLIg:pFUSE-CHIg plasmids. pFUSE2-CLIg plasmids feature the constant region of a kappa or lambda light chain. pFUSE2-CLIg plasmids are selectable with blasticidin. pFUSE-CHIg plasmids are selectable with Zeocin®.

Antibody generation using pFUSE-CHIg & pFUSE-CLIg

1- Obtention of VH and VL sequences



2- Cloning into pFUSE-CHIg and pFUSE-CLIg



To check for production of your antibody after transfection, you may take an aliquot of growth medium and perform SDS-PAGE, protein-specific ELISA, or the bioactivity assay of choice to determine that your cells are producing your antibody of interest.

The resulting IgA antibody that can be purified from the supernatant using the appropriate affinity chromatography.

RELATED PRODUCTS

Product	Catalog Code
pFUSE2-CLIg-mk	pfuse2-mclk
pFUSE2-CLIg-ml1	pfuse2-mcll1
pFUSE2-CLIg-ml2	pfuse2-mcll2
LyoVec™	lyec-12
Protein L / Agarose	gel-protl-2
Zeocin®	ant-zn-1

TECHNICAL SUPPORT

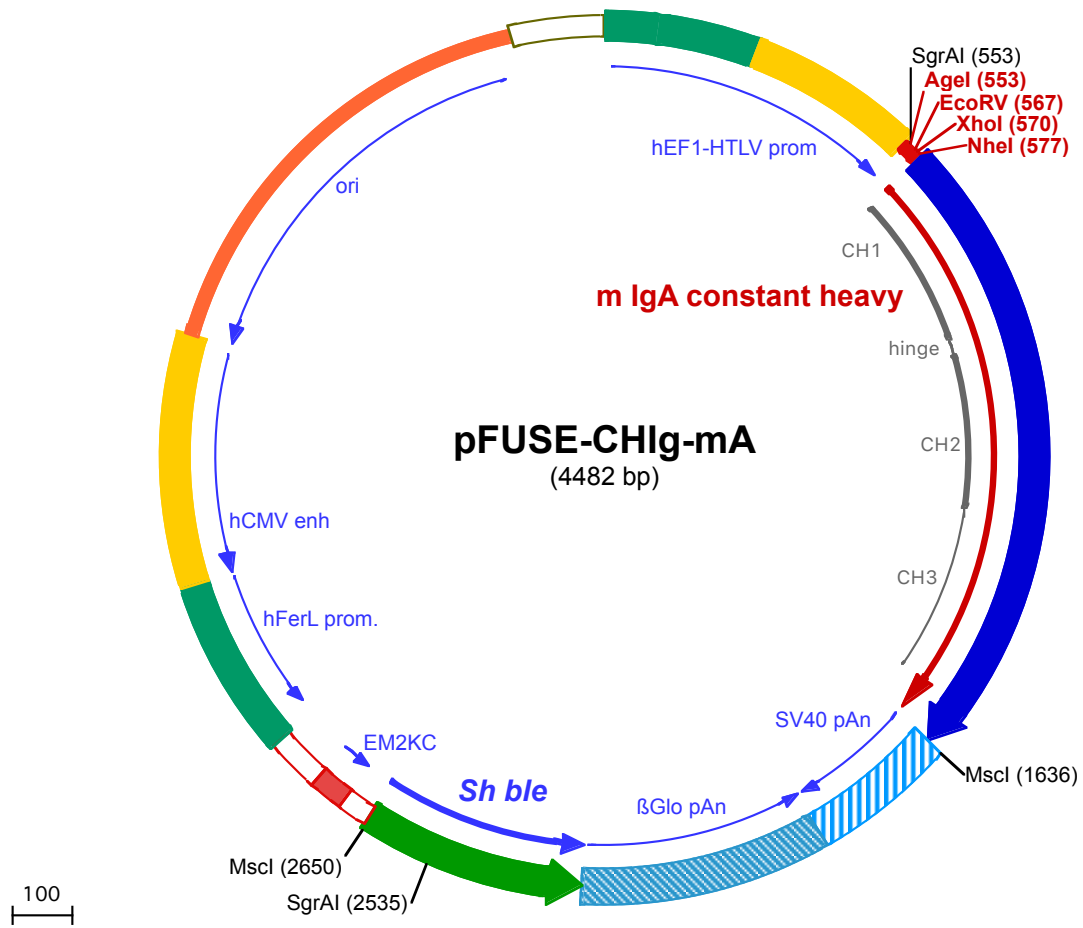
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InvivoGen USA (International): +1 (858) 457-5873

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InvivoGen Asia: +852 3622-3480

E-mail: info@invivogen.com



1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGAGAGCGCACATGCCACAGTCCCGGAGAAGTTGGGGGAGGGTGGCAATTGAACGGGTGCCTA

101 GAGAAGGTGGCGCGGGTAAACTGGGAAAGTGATGTCGTGACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

201 GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTCACGCGCCCGCCCTACCTGAGGCC

301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCTCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC

401 GGGCCTTTGTCCGGCGCTCCCTTGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTGCCTGACCTGCTTGTCTCAACTCTACGCTTTGTTTCGTTT

501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGCGCCTACCTGAGATCACCGGTGAATTCGATATCTCGAGTGTAGCTCAGAGTCTGCGAGAAT

Agel (553) XhoI (570)
 SgrAI (553) EcoRV (567) NheI (577)

1 ▶ S S E S A R N

601 CCCACCCTACCCACTGACACTCCCACCAGCTCTGTCAAGTGACCCAGTGATAATCGGTGCTGCTGATTACGATTACTTCCCTTCCGGCACGATGAATG

8▶ P T I Y P L T L P P A L S S D P V I I G C L I H D Y F P S G T M N

701 TGACCTGGGAAAGAGTGGGAAGGATATAACCACCGTAAACTCCACCTGCCCTGGCTCTGGGGACGGTACACCATGAGCAGCCAGTTGACCCTGCC

41▶ V T W G K S G K D I T T V N F P P A L A S G G R Y T M S S Q L T L P

801 AGCTGTCGAGTGCCAGAAGGAGAATCCGTGAAATGTTCCGTGCAACATGACTCTAACCCCGTCCAAGAATTGGATGTGAATTGCTCTGGTCTACTCCT

74▶ A V E C P E G E S V K C S V Q H D S N P V Q E L D V N C S G P T P

901 CCTCTCTATTACTATTCTTCTGCCAGCCAGCCTGTCACTGCAGCGCCAGCTCTTGAGGACCTGCTCTGGGTTGAGATGCCAGCATCACATGTA

108▶ P P P I T I P S C Q P S L S L Q R P A L E D L L L G S D A S I T C

1001 CTCTGAATGGCCTGAGAAATCCTGAGGGAGCTGTCTTACCTGGGAGCCCTCCACTGGGAAGGATGCAGTGCAGAAGAAAGCTGTGCAGAATTCCTGCGG

141▶ T L N G L R N P E G A V F T W E P S T G K D A V Q K K A V Q N S C G

1101 CTGCTACAGTGTGTCAGCGTCTGCTGGCTGTGCTGAGCGTGGAAACAGTGGCGCATCATTCAAGTGCACAGTTACCCATCCTGAGTCTGGCACCTTA

174▶ C Y S V S S V L P G C A E R W N S G A S F K C T V T H P E S G T L

1201 ACTGGACAATTGCCAAAGTACAGTGAACACCTTCCACCCAGGTCCACCTGCTACCGCCCGCTCGGAGGAGTGGCCCTGAATGAGCTCTTGTCCC

208▶ T G T I A K V T V N T F P P Q V H L L P P P S E E L A L N E L L S

1301 TGACATGCTGGTGCAGCTTTCAACCTAAAGAAGTGTGGTGCATGGCTGCATGGAAATGAGGAGCTGTCCCAGAAAGTACCTAGTGTGTTGAGCC

241▶ L T C L V R A F N P K E V L V R W L H G N E E L S P E S Y L V F E P

1401 CCTAAAGGAGCCAGGCGAGGAGCCACCACCTACCTGGTGACAAGCGTGTGCGTGTATCAGTGAACCTGGAAACAGGGTACCAGTACTCCTGCATG

274▶ L K E P G E G A T T Y L V T S V L R V S A E T W K Q G D Q Y S C M

1501 GTGGGCCACGAGGCTTCCCATGAACTTACCAGAAGACCATGACCGTCTGTCGGTAAACCCACCAATGTCAGCGTGTCTGTGATCATGTCAGAGG

308▶ V G H E A L P M N F T Q K T I D R L S G K P T N V S V S V I M S E

MscI (1636)

1601 GAGATGGCATCTGCTACTGAGCCACCCTCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAACCAAT

341▶ G D G I C Y •

1701 GCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACCAATTGCATTATTATGTTTCA

1801 GGTTCCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTAATTCTAAAATACAGCATAGCAAACTTTAACCT

1901 CCAAATCAAGCCTCTACTTGAATCCTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGACGCCTCACCTT

2001 CTTTCATGGAGTTTAAGATATAGTGTATTTTCCAAGGTTTGAAGTACTCTTCAATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTTTA

2101 GTAAAATATTAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCC

2201 AGTTTAGTAGTTGGACTTAGGGAACAAAGAACCTTTAATAGAAATGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCTGCTCTCTGCCAC

125 ◀ • D Q E E A V

2301 AAAGTGCACGCAGTTGCCGGCCGGTTCGCGCAGGGCGAACTCCCGCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCCGGAGGCGTCCCGGAAG

118▶ F H V C N G A P D R L A F E R G W P Q E G I E T M A P G S A D R F

2401 TTCGTGGACACGACTCCGACACTCGGCGTACAGCTCGTCCAGGCGCGCACCCACCCAGGCCAGGGTGTGTCCGGCACCACCTGGTCTGACCCG

84▶ N T S V V E S W E A Y L E D L G R V W V W A L T N D P V V Q D Q V A

SgrAI (2535)

2501 CGCTGATGAACAGGGTACGTCGTCGCCGACACCCGGCAAGTCTCTCCACGAAGTCCCGGAGAACCCGAGCCGGTCCGATCCAGAACTCGACCCG

51▶ S I F L T V D D R V V G A F D D E V F D R S F G L R D T W F E V A

MscI (2650)

2601 TCCGGCGACGTCGCGCGCGGTGAGCACCGAACGGCACTGGTCAACTTGGCCATGATGGCTCCTCctgtcaggagaggaagagaagaaggtagtaca
 18 G A V D R A T L V P V A S T L K A M
 2701 ttgCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGATTAATTGTCAAAGTACAGGGCTGCAgggttcatagtgccacttttcctgactg
 2801 ccccatctcctgcccaccctttcccaggcatagacagtcagtgacttacCAAAGTACAGGAGGGAGAAGGCAGAAGCTTGAGACAGACCCGCGGGACCG
 2901 CCGAACTGCGAGGGGACGTGGCTAGGGCGGCTTCTTTTATGGTGCGCCGCCCTCGGAGGCAGGGCGCTCGGGGAGGCCTAGCGGCAATCTGCGGTGGC
 3001 AGGAGGCGGGGCCAAGGCCGTGCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCGCCCAAGCAAGGGGAAGTCACGCGCCTGTAGCGCCAG
 3101 CGTGTGTGAAATGGGGCTTGGGGGGTGGGGCCCTGACTAGTCAAAACAACTCCATTGACGTCAATGGGGTGGAGACTTGAAATCCCGTGAGT
 3201 CAAACCGTATCCACGCCATTGATGTACTGCCAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCA
 3301 TAAGGTCACTGACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCTACTTGGCATATGATACTTGTACTGCAAG
 3401 TGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCAATTATTGACGTCAATGGGCGGG
 3501 GGTCGTTGGGCGGTGAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAGGCCAGCAAAGGCCAGG
 3601 AACCGTAAAAAGGCCGCTTGTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGA
 3701 CAGGACTATAAAGATACCAGGCTTTCCCTGGAAGCTCCCTCGTGCCTCTCTGTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCC
 3801 TTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCAAGCTGGGCTGTGTGCACGAACCCCGTT
 3901 CAGCCCGACCGCTGCGCCTTATCCGTAAGTATCGTCTTGTGAGTCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTA
 4001 GCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAA
 4101 GCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTGTAAGCAGCAGATTACGCGC
 4201 AGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGAACGAAAAGTACAGTTAAGGGATTTTGGTATGGCTAGTT
 4301 AATTAACATTTAAATCAGCGGCCGAATAAAATATCTTTATTTTATTACATCTGTGTGGTTTTTTGTGTAATCGTAACATAACATACGCTCTCCAT
 4401 CAAAACAAAACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTATCGAA